A

SYNOPSIS

On

Studies On Effect of Antibiotics And Herbal Extract Against Nosocomial Infections Causing Micro-organisms

Proposed work plan for Ph.D.degree

Submitted

To

SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED

स्वामी रामानंद तीर्थ
मराठवाडा विद्यापीठ,नांदेड.

For The

Research Leading to Ph.D. Degree

in the subject of

Microbiology

by

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Year-2018

Introduction:-

Nosocomial infections are also called as healthcare acquired infection. It is defined by the Disease Control and Prevention (CDC) is a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or toxin(s), without any evidence that the infection was present or incubating at the time of admission to the acute care setting **(Pradhan *et al* 2014).**Nosocomial (from the Latin nosocomium meaning hospital) infections are infections in acquired in that were neither present nor incubating at the time of the patient's admission to hospital **(Jenkins *et al* 2017).** The importance of *Staphylococcus aureus* as a persistent nosocomial and community acquired pathogen has become a global health concern. It has a remarkable capability of evolving different mechanisms of resistance to most antimicrobial agents **(Onwubiko *et al* 2011).**Nosocomial or hospital acquired infections are defined as infections which are acquired during the hospital stay. Nosocomial infections are usually those infections that are identified at least 48–72 hours following admission to health institutions. The common bacterial pathogens present in the blood stream infection (BSIs) and urinary tract infection (UTIs) are *Staphylococcus aureus,* Coagulase-Negative *Staphylococci* (CoNS), *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp., *Enterococcus* spp., and *Acinetobacter* spp. **(Ghadiri *et al* 2012).**Surgical site infections (SSIs) are one of the most common health care associated infections and cause significant morbidity, increase in hospital stay, and increase in treatment costs. The rate of SSI for all surgical procedures vary between 9% and 23% and found that methicillin-sensitive *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* are the most common cause of SSI. **(Shah *et al* 2015).**

Extended-spectrum beta lactamase (ESBL) producing and carbapenem resistant Enterobacteriaceae (CRE), methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus species,* and multidrug-resistant *Acinetobacter baumannii* are associated with both hospital-acquired and community-acquired infections. Antimicrobial-resistant pathogens that are associated with nosocomial infections, or health care–associated infections (HAI) pose an ongoing challenge to hospitals, both in terms of patient treatment and in the prevention of transmission of resistant pathogens from patient to patient **(Denys *et al* 2014).**

Review of Literature:-

International:-

The wider use of more aggressive modalities of treatments, such as hematopoietic stem cell transplantation (HSCT), solid organ transplantation (SOT), new chemotherapeutic agents, and immunomodulatory agents, has increased the population of immunocompromised patients at risk for invasive fungal infection.The prevalence of invasive devices, especially intravascular central lines, has resulted in an increase in nosocomial catheter-related bloodstream infections (CRBSIs), candidemia, and disseminated candidiasis.*Candida spp* were the fourth most common pathogen accounting for 11% of 28,502 HAIs reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention (CDC)(**Alangaden *et al* 2011).**

Candida spp are the most common fungal pathogens causing serious HAIs, especially in patients admitted to intensive care units (ICUs) worldwide. Certain *Candida spp*, especially *Candida albicans*, are part of the human microbial flora; hence, most candidal infections are endogenous in origin. Invasive candidiasis, namely, candidemia, disseminated hematogenous infections, or deep-seated infections in normally sterile body sites, can occur in immunocompromised patients, such as those with neutropenia, and in critically ill patients. **(Alangaden *et al* 2011).**

Nosocomial infections (NIs) are one of the major causes of mortality and morbidity in the neonatal intensive care units (NICUs). Nosocomial infections (NIs) is an infection during hospitalization that was not present or in incubation at the time of admission that has an impact on the healthcare system as it increases the use of medical resources, duration of hospitalization, as well as increased cost of treatment in both developed and developing countries. These are major public health problems worldwide, but particularly in developing countries. The incidence of infections varies widely among NICUs. It occurs at an incidence of around 30% and in the developing countries, it is estimated to cause 40% of all neonatal deaths, depending on environmental factors and differences in clinical practice **(Mohammed *et al* 2014).**

Bacteria are the most common pathogens responsible for nosocomial infections. Some belong to natural flora of the patient and cause infection only when the immune system of the patient becomes prone to infections. *Acinetobacter* is pathogenic bacteria responsible for

infections occurring in ICUs. *Bacteroides fragilis* is a commensal bacteria found in intestinal tract and colon. Methicillin-resistant *Staphylococcus aureus* (MRSA) transmit through direct contact, open wounds and contaminated hands. It causes sepsis, pneumonia and SSI by travelling from organs to bloodstream. It is highly resistant towards antibiotics called beta-lactams **(Busl *et al* 2017).**

Nosocomial infections are an important factor in the emergence and spread of multidrug-resistant (MDR) bacteria. Broad spectrum antibiotics, such as vancomycin, third-generation cephalosporins and carbapenems, are often used for empirical treatment of infected patients, thereby selecting for and favoring the persistence of MDR pathogens **(Jenkins *et al* 2017).** Health care associated infections (HAIs) pose a major threat for patients and health care workers, particularly in intensive care units (ICUs).Surveillance has been recognized as the cornerstone of effective HAI prevention and control programs. The standardized criteria for HAIs surveillance in the acute care setting have been established by the Centers for Disease Control and Prevention's (CDC's) previous National Nosocomial Infection Surveillance System (NNISS) **(Peng *et al* 2015).**

National:-

Surgical Site Infections (SSI) is nosocomial infections be fall in 2-5% of patients subjected to surgery. These are the second most common type of nosocomial infections mainly caused by *Staphylococcus aureus* **(Khan *et al* 2017).** Several strains of *Escherichia coli* (*E. coli*) are quite harmless and colonize the healthy human bowel, but recently developed strains are pathogenic causing frequently, cholecystitis, bacteremia, cholangitis, urinary tract infections (UTI), traveler's diarrhoea, neonatal meningitis, pneumonia, liver abscess and a few more **(Rath *et al* 2014).**Most common are pneumonia, urinary tract infections (UTIs), bloodstream infections, and intracranial infections (meningitis and ventriculitis).Ventilator-associated pneumonia (VAP), defined as pneumonia in patients undergoing mechanical ventilation for at least 48 hours, develops because of aspiration of contaminated oropharyngeal secretions around the endo tracheal cuff. **(Khan *et al* 2017).** *Staphylococcus aureus* is the leading cause of a variety of hospital infections, ranging from minor skin infections to fatal septicemia. *S. aureus* is known for its ability to develop resistance to antibiotics. An infection caused by it is used to respond to ß-lactam and related group of antibiotics, but the emergence of methicillin-resistant *S. aureus* (MRSA) has posed a serious therapeutic challenge. The possible predisposing factors that increase the chance of

emergence and spread of MRSA are prolonged and repeated hospitalization, indiscriminate use of antibiotics, and lack of awareness, intravenous drug abuse, and presence of indwelling medical devices **(Sharma *et al* 2013).** Central line associated bloodstream infections (CLABSI) are deadly nosocomial infections with the death incidence rate of 12%-25%. Catheter associated urinary tract infections (CAUTI) is the most usual type of nosocomial infection globally. CAUTI can develop to complications such as, orchitis, epididymitis and prostatitis in males, and pyelonephritis, cystitis and meningitis in all patients **(Khan *et al* 2017).**Health care associated infection is a key factor determining clinical outcome among patients admitted in critical care areas. Surveillance of device associated infections has become an integral feature of infection control in all hospitals **(Datta *et al* 2014).** Hospital-acquired infections (HAI) are a significant cause of increased morbidity and mortality in hospitalized patients.Hospital-acquired infections HAI are a cause of prolonged hospital stay, are inconvenient for the patient, and constitute an economic burden on health care .It is estimated that 80% of all hospital deaths are directly or indirectly related to HAIs **(Malhotra *et al* 2014)**.

Regional Study:-

Candida blood stream infection (BSI) has become a major problem in tertiary care hospital worldwide. Despite some improvement in fungal BSI diagnosis during recent years, diagnosis of candidemia remains difficult. Candidemia has been associated with many risk factors like long- term hospitalization, antibiotic therapy, use of intravascular catheters, and underlying diseases like diabetes and malignancy **(Deorukhkar *et al* 2012).**Neonatal deaths account for over a one third of the global burden of child mortality.Nosocomial infections are one of the most important causes of mortality and morbidity in hospitals; particularly in developing countries. Nosocomial infections have common frequency in pediatrics wards and represent one of the major causes of morbidity in the neonatal intensive care units (NICUs). **(Naregal *et al* 2015).** Now-a-days mobile phone utilization has increased in health care system and its acceptance by health care personnel has increased. Whether mobile phones can transmit the infections Sources of infections of mobile phones include hands of health care workers, inanimate objects like bed, instruments & furniture etc. ands of the Doctors and health care personnel play important role in transmission of hospital acquired infections (HAIS) **(Tankhiwale *et al* 2012).**Maximum scientist and research was studied on only Intensive care unit (ICUs) and neonatal intensive care units (NICUs) in abroad as well as in India. Very less

study was done in Maharashtra that's why I will decide to study on female ward and tuberculosis ward.

Types of nosocomial infections:-

National Healthcare Safety Network with Center for Disease Control (CDC) for surveillance has classified nosocomial infection sites into 13 types, with 50 infection sites, which are specific on the basis of biological and clinical criteria. The sites which are common include urinary tract infections (UTI), surgical and soft tissue infections, gastroenteritis, meningitis and respiratory infections. A change regarding nosocomial infection sites can be easily detected with time due to the elevated use of cancer chemotherapy, advancement in organ transplantation, immunotherapy and invasive techniques for diagnostic and therapeutic purposes. The perfect example of this can be seen in the case of pneumonia as prevalence of nosocomial pneumonia increased from 17% to 30% during five years **(Khan *et al* 2015).**

Agents of nosocomial infections:-

Nosocomial infections are caused by many microbes and each one can cause infection in healthcare settings. Bacteria are responsible for about ninety percent infections, whereas protozoans, fungi, viruses and mycobacteria are less contributing compared to bacterial infections **(Khan *et al* 2015).**The agents that are usually involved in hospital-acquired infections include *Streptococcus spp., Acinetobacter spp*., enterococci, *Pseudomonas aeruginosa* (*P. aeruginosa*), coagulase-negative *staphylococci, Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Legionella* and Enterobacteriaceae family members including *Proteus mirablis, Klebsiella pneumonia* (K. pneumonia), *Escherichia coli* (E. coli), *Serratia marcescens*. Out of these enterococci, *P. aeruginosa, S. aureus* and *E. coli* have a major role **(Horan *et al* 2008).** UTI usually contain *E. coli*, while it is uncommon in other infection sites. Contrarily, *S. aureus* is frequent at other body sites and rarely causes UTI. In blood-borne infections, coagulase-negative *S. aureus* is the main causative agent. Surgical-site infections contain *Enterococcus spp*. which is less prevalent at respiratory tract**(Khan *et al* 2015).**Proteus spp.,*Klebsiella spp*. and *Escherichia spp.* were responsible for nosocomial infections in the 1960s, but from 1975 to 1980s, *Acinetobacter spp.* with *P. aeruginosa* created clinical difficulties **(Gordon *et al* 2008).** During the recent years, *streptococci* along with coagulase-negative *staphylococci* and coagulase-positive *staphylococci* reemerged and incidence level of

K. pneumonia and *E. coli* declined from 7% to 5% and 23%–16%, respectively **(Klein *et al* 2007).**

Nosocomial Pathogens:-

A multicenter study was conducted in Japan to isolate bacteria from surgical infections during 2011–2012. About 785 strains including 31 of *Candida spp*. were isolated from 204 out of 259 surgical patients. About 523 strains were isolated from primary infections and 231 from surgical site infection. From primary infections, anaerobic Gram-negative bacteria were prevalent. *Enterococcus spp*. was the highest among Gram positive aerobic bacteria followed by *Streptococcus* and *Staphylococcus spp. E. coli* was the predominant form among the Gram-negative aerobic bacteria followed by *K. pneumonia, P. aeruginosa* and *Enterobacter cloacae* **(Shinagawa *et al* 2014).**

S. aureus

Out of many species of *Staphylococcus genus, S. aureus* is considered one of the most important pathogens, responsible for nosocomial infections. It is Gram-positive cocci, non-spore forming, catalase- and coagulase-positive, immotile, facultatively anaerobe **(Vandenesch *et al* 2012).**Hospitalized patients with decreased immunity and immunocompetent people in community are more prone to *S. aureus* infections. *S. aureus* infects not only the superficial but also the deep tissues and local abscess lesion **(Laham *et al* 2015)** and exfoliative toxins cause *staphylococcal* scalded skin syndrome. Virulence mechanisms of *S. aureus* include toxins, enzymes and immune modulators **(Vandenesch *et al* 2012).**

E. coli

E. coli is an emerging nosocomial pathogen causing problems in health care settings **(Lausch *et al* 2013).***E. coli* is Gram-negative and oxidase-negative facultative anaerobe bacteria. It can colonize in gastrointestinal tract of human beings and other animals. *E. coli* is responsible for a number of diseases including UTI, septicemia, pneumonia, neonatal meningitis, peritonitis and gastroenteritis **(Zhao *et al* 2015).**Specialized virulence factors are seen in case of UTI and gastroenteritis **(Khan *et al* 2015).**

Vancomycin-resistant enterococci

Enterococci are the second leading cause of hospital acquired infections worldwide and the main leading cause in United States contributing 20%–30% of infections. These are facultative anaerobic Gram-positive enteric microbes **(Karki *et al* 2015).**Enterococci are involved in the blood-borne infections; UTI and wound infections consort to surgical procedures **(Kaiser *et al* 2015).** Virulence factors include extracellular surface proteins, cytolysin, adhesions, hemolysins, gelatinase, extracellular superoxide and aggregation substances **(Sood *et al* 2008).**

K. pneumonia

Three to seven percent of hospital-acquired bacterial infections are related to *K. pneumonia*, which is the eighth significant pathogen in healthcare settings. It is a Gram-positive bacillus and an opportunistic bacterium, which is a part of Enterobacteriaceae family. It usually colonizes gastrointestinal tract, pharynx and skin. It gets involved in diseases such as neonatal septicaemia, pneumonia, wound infections and septicemia. Its virulence factors include endotoxins, cell wall receptors and capsular polysaccharide **(Lin *et al* 2015).**

P. aeruginosa

P. aeruginosa contributes to 11% of all nosocomial infections, which result in high mortality and morbidity rates. It is non-fermenter Gram-negative organism causing diseases especially among immune-compromised people. The sites of colonization are kidney, urinary tract and upper respiratory tract. It is a cause of surgical and wound infections, UTI, pneumonia, cystic fibrosis and bacteremia. Some of important virulence factors are adhesions, hemolysins, exotoxins, proteases and siderophores **(Balasoiu *et al* 2014).**

Clostridium difficile (C. difficile)

C. difficile is an important nosocomial pathogen which mainly causes diarrhea. Several cases of *C. difficile* are reported in Europe, U.S. and Canada. It is a Gram-positive bacillus. It is anaerobic and spore-forming bacteria. It usually colonizes in intestinal tract and serves as part of normal microbiota.Diseases caused by toxins produced by *C. difficile* are colitis

and it is responsible for 15%–25% cases of diarrhea. Major virulence factors for *C. difficile* are toxins, fimbriae, capsule and hydrolytic enzymes **(Khan *et al* 2015).**

Aims and objectives:-

A) To collect samples from female ward (FMW) & tuberculosis ward (TBW).
B) To isolate causative organism from collected clinical samples responsible for nosocomial infection.
C) To study and identify the biochemical characteristics of the isolated pathogens by FAME (Fatty Acid Methyl Ester).
D) To study antibiogram among clinical isolates and establishment of MIC values.
E) To study synergetic action of antibiotics against antibiotic resistant isolates.

Year wise Plan:-

Ist year: - Literature survey (National and International) collection of clinical specimen, enrichment, Isolation and identification of respective pathogen on selective media.

IInd year: - Antibiotics sensitive testing will be done by bioassay methods.

- Isolated pathogens will be identified.
- Synergetic action of different antibiotics on resistant bacteria will be studied.
- Result will be complied.
- Final synopsis will be prepared.
- Thesis writing.

Materials and Method:-

A) Collection of clinical samples

Various clinical specimens such as swab of bed, swabs from saline stands, ventilator machines, wound swab, blood culture, umbilical cord swab, urine, eye swab, ear swab, abscess, catheter tips, throat swab, pleural aspirate and skin swab will be collected from female ward (FMW) and tuberculosis ward (TBW) in pre-sterilized containers from indoor patient departments of Civil Hospital, Parbhani, of the Maharashtra state of India. For transportation of samples I will use transport media will be such as Selenite F broth, Stuart Transport Medium, Cary Blair medium, thioglycolate broth and buffered saline solution.

B) Isolation and Identification

Various clinical specimens such as wound swab, blood culture, umbilical cord swab, urine, eye swab, ear swab, abscess, catheter tips, throat swab, pleural aspirate and skin swab.Isolation of nosocomial infection causing microorganism will be done on all ship blood agar, chocolate agar, and Mac Conkey agar plates, eosin methylene blue agar, Blood agar, manitol salt agar, cysteine deoxycholate electrolyte deficient (CLED) agar (HiMedia, Mumbai). Baird-Parker agar (BD, Heidelberg, Germany), for 24 hour at 37^{O}C. Colonies will be streaked on respectively agar (HiMedia Lab. Ltd., Mumbai, India). Plates will be incubated at 35^{O}C ± 2^{O}C for 24 hours. **(Ghadiri *et al* 2012).**Carbohydrate assimilation test, growth and fermentation profile on various sugars will be done for primary conformation of organism. All pure cultures will be maintained on respective agars slants, at 4^{O}C temperature. Identification of nosocomial pathogen will be done on FAME.

Antibiotic Susceptibility Testing.

Antibiotic susceptibility testing will be performed by Kirby-Bauer's disk diffusion method on Muller-Hinton agar (Hi Media, Mumbai, India) in accordance with the standards of the Clinical Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards [NCCLS]) guidelines **(Ghadiri *et al* 2012).**calculation of antibiogram will be done by CLSI guidelines.

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